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Chemical and Microstructure Characteristics of Dangke at Various of Temperature Ripened

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Abstract : The current study was highlighted the use of oleaginous fungi isolated from different Iraqi ecosystems (polluted water and soil)as raw material for production of myco-diesel or biodiesel which can be used as alternative to fossil fuels , many countries are currently trying to find sources for this type of diesel in preparation for the post- petroleum phase because it is low costand eco-friendly .Oleaginous fungal isolates grown on liquid natural medium date syrup and it is possible to use as a substrate for cultivating of oleaginous fungal isolates , the results showed good biomass of the dry weight was the highest value 10.22 g / L and the highest yield of lipids was 5.35 g / L to *A.terreus*on date syrup medium . Fatty Acid Methyl Asters FAMEs compositions were mainly composed of long chain saturated and unsaturated fatty acids, decanoic, tridecanoic, pentadecanoic, hexadecanoic acid in high amounts that reached 34%. , then Octadecenoic acids 15% and 11- , Octadecenoic acids 10% and few amounts of decanoic and tridecanoic .The oleaginous fungal test *A.terreus* was tested for its ability to increase lipid accumulation intracellular cells before and after exposing of U.V. light , the results revealed increasing of exposing of U.V light for 5 min. and 10 min. of spore suspension of *A.terreus* before cultivating on liquid medium
Keywords : Oleaginous fungi – mycodiesel –U.V mutagenesis –date syrub.

Introduction

Petroleum-based fuels have been the main energy source of modern society since the Industrial revolution. Due to increasing energy demands; we are facing the risk of an energy shortage due to decreasing reserves of fossil fuels. Numerous governmental and industrial efforts are therefore focused on exploring alternative energy sources that are renewable and carbon neutralone of these alternative energy sources is biodiesel derived from microbes specially fungi which can accumulate significant amounts of lipid[1]. Fungi metabolically transform the external carbon into carbohydrate or hydrocarbon and then to lipids which are considered to be important storage compounds in the form of Triacylglycerols (TAG)[2 ´3].If the lipid content in the cell exceeds 20% or more of their cell mass being composed of lipids biomass then microorganism can be called as oleaginous. Therefore, oleaginous filamentous fungi are suggested as a favorable feedstock for a sustainable biodiesel industry and it seems to be a good alternative for fossil fuels[4].

Many fungal species are able to accumulate lipids, including *Aspergillus oryzae,Mortierella isabellina, Mortierella alliacea, Humicola lanuginose, Trichoderma reesei, Mortierella vinacea* and *Mucorcircinelloides*[5,

[6]. Isolated strains of, *Fusarium graminearum Aspergillus terreus, Aspergillus flavus* and *Aspergillus fumigatus Trichoderma* spp., *Mucor circinelloides* and *Gliocladium roseum* also are reported as potential oil accumulators[7, 8, 9].

The purpose of this project focusing on utilization of biodiesel or mycodiesel as alternative to fossil fuel which are needed to displace petroleum derived transport fuels specially the fossil fuel could gone before the end of century through exploitation the microbes (fungi) for biodiesel production and to optimize the conditions for higher lipids productions from oleaginous fungi.

Material and methods

Samples Collection for Isolation of Oleaginous Fungi from Different Environmental Sources

Fungi were isolated from polluted soil and polluted water by waste oil of generators from different locations at Baghdad –Iraq , serial dilutions of the collected samples was carried out Olutiola and others[10] and 1 ml of the dilute was pour plated on Potato Dextrose agar (PDA) supplemented with chloramphenicol, three dilutes were used in this study in triplicates. PDA plates were incubated at 28 ºC for 3-6 days. Morphological appearances of the inoculated plates (at room temperature) were observed and distinct colonies were sub-cultured to obtain pure isolates which were then maintained on PDA slants and stored at 4ºC for further study.

Screening Fungi Isolated for Lipid Production

To select the highest lipid producer among them, isolates were cultured in basal medium containing a chromogenic substrate (Congo red) was used to screen the isolates for lipase production. (in g/L:,10g peptone, 5g NaCl, 0.1g Calcium chloride ,1ml castor oil, 15g agar, 0.5g Congo red with initial pH 6.0).The sterile medium was prepared and poured plates and allows solidifying. An agar plug (5 mm) was removed from the periphery of 7 days old cultures grown on PDA plates of each fungus , then placed in the center culture medium plates on to triplicate plates containing the screening medium , plates were incubated at 25°C until the fungal growth for 14 days in an incubator . Lipolysis was indicated by the appearance of clear zone of inhibition around the disc of inoculation. The diameters of the colonies and clearance zones were measured after 14 days [11,12].

Test the Efficiency of date of syrup medium for Biomass and lipid production by Oleaginous Fungi

Preparation the natural medium of date of syrup after the cutting to small pieces of fruit of date *Phoenix dactylifera* and 20 gm. added to the amount of distilled water and boiled for 30 minutes and cooled and put in a mixer blender for 5 minutes and filtered using medical gauze and complements the volume supernatant of distilled water to 1000ml and adjusted the pH to 6.0 and each500ml medium were filled in 1000 ml erlenmeyer flask and sterilized in autoclave (121℃ and under 15 lbs/In^2 pressure for 20 minute) cooled then inoculated by adding5 discs fungal isolates incubated for 14days at 30 ºC, then followed the extraction of biomass and dry weight of 6 selected oleaginous fungal isolates and estimated the dry weight according to [13]. and lipid production in the same of methods that were followed in [8].

Biodiesel Production and Analysis by Gas Chromatography

Total lipids were extracted from the dried biomass from the previous items. The fatty acids compositions of the lipid produced by oleaginous fungal isolates were determined by analysis of Fatty Acid Methyl Esters (FAMEs) depended upon the method of [14]with some modification. The FAMEs were produced by transesterification reaction. 2 ml of methanol with sulfuric acid (2.5% V/V H2SO4/CH3OH) as a catalyst was added to the crude lipid 100 mg. The reaction was progressed for 45 min at 90º C (water bath). Then, 1 mL H2O and 2 mL n-hexane were added. The FAMEs were dissolved into n-hexane. The solution was centrifuged at 2000 rpm for 15 min to compact the water from the hexane phase containing FAMEs, which was then transferred into glass vials by using Pasteur pipettes. The FAMEs in n-hexane were analyzed using a gas chromatograph after adding 0.1ml of solution (KOH, methanol 1% W/ V) and 1ml heptane to The FAMEs . A gas chromatography (GC) analyzer (GC-17A model by Shimadzu Inc., Kyoto, Japan) with the Chromatography Data Management system was used to analyze the weight proportions of the fatty acids.

Lipids production on basal medium by fungal test *Aspergillus terreus* before and after mutation

The oleaginous fungal isolate test *Aspergillus terreus* used in this study selected for the best productive isolates of lipids was exposed to U.V. light in (Dispensing –Cabinet) for different periods of time 5 min and 10 min at the distance of 20 cm from the U.V source . Each U.V exposed

Spore suspension was stored in dark overnight to avoid photo reactivation. U.V treated fungal spore suspension of 1 ml and spore suspension untreated (control) were inoculated into liquid medium 100 ml of Yeast Extract Glucose medium incubated for 14days at 30 ºC, then followed the extraction of Biomass and dry weight of 6 selected oleaginous fungal isolates and estimated the dry weight according to(13), and lipid production in the same of methods that are followed in (8).

Results and Discussion

Isolation of fungi from different types of environments fungal isolates with different visible colony and cell morphology (as seen under the microscope) were obtained from polluted soil and polluted water by waste oils of generators from different locations at Baghdad –Iraq, of which 14 and 15 were yeasts, while 14and 36 were filamentous fungi from polluted soil and water.

Screening Oleaginous Fungi Isolated for Lipase production on Solid Agar

The screening of fungal isolates for lipase production on screening lipase medium is shown in Table 1 The lipolytic activity was indicated by the appearance of clear zone of inhibition around the disc of inoculation after 14 days of incubation. The results showed the ability of fungal isolates to produce lipase ranged within producer ,weak producer and non-producer .

Table 1: Screening of oleaginous fungal isolates for lipase production on solid agar. The symbol (+) producer and (-) non producer

Source	Type of fungi	Results for lipase production
Soil polluted	*Aspergillus terreus*	+
	Trichoderma harzianum	+
	Aspergillus terreus	+
	Penicillium sp.	+
	Aspergillus flavus	+
	Candida sp.	_
	Areobasidium sp.	_
	Rhodotorula sp.	_
	Aspergillus fumigatus	_
Water polluted	*Candida* sp.	_
	Fusarium oysporum	+
	Aspergillus terreus	+
	Aspergillus niger	Weak producer
	*Aspergillus flav*us	Weak producer
	Rhodotorula sp.	_

Microbial lipases are currently receiving much attention because of their biotechnological potential, such as broad substrate specificity, high yield and low cost production and so on. Therefore, they have been widely used in industrial applications, such as biodiesel production, organic synthesis, food, pharmaceutical, and detergents chemistry [15,16] . It could thus be inferred that there was an increase in lipase production by some of the filamentous fungal isolates This is in agreement with study of[17.]

Biomass Production and Lipids Extraction

The highest bio-mass and lipid yield of selected oleaginous fungal isolates , fungal isolates grew well in 14 days in natural liquid medium date of syrup and showed good biomass under the given carbon rich, nitrogen limiting conditions. The highest value10.22 g / L (Figure -1). And the total lipid from the biomass was extracted and estimated (Figure -2) the highest yield of lipids was 5.35 g / L to *A.terreus*.

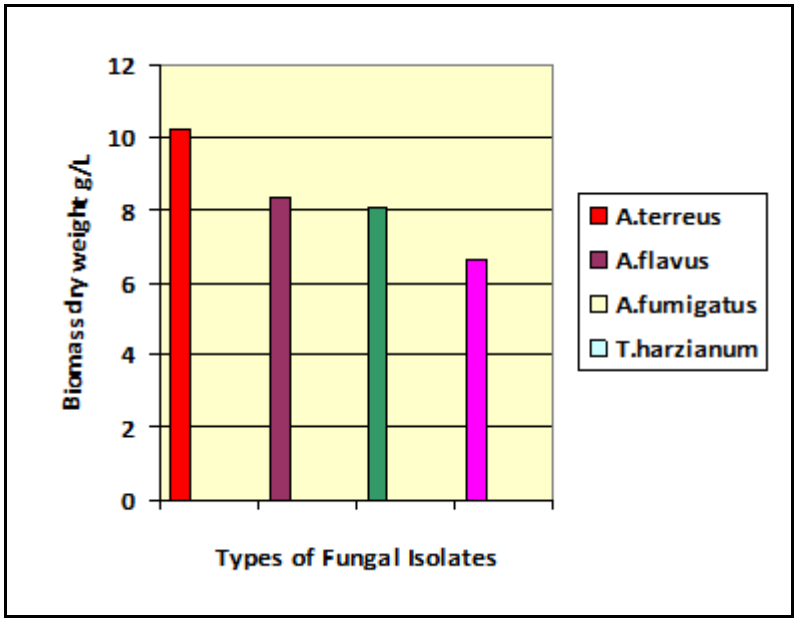

Figure 1. Fungal biomass of dry weight in syrup date medium , pH 7and incubated for 7 days, 120 rpm at 30˚C.

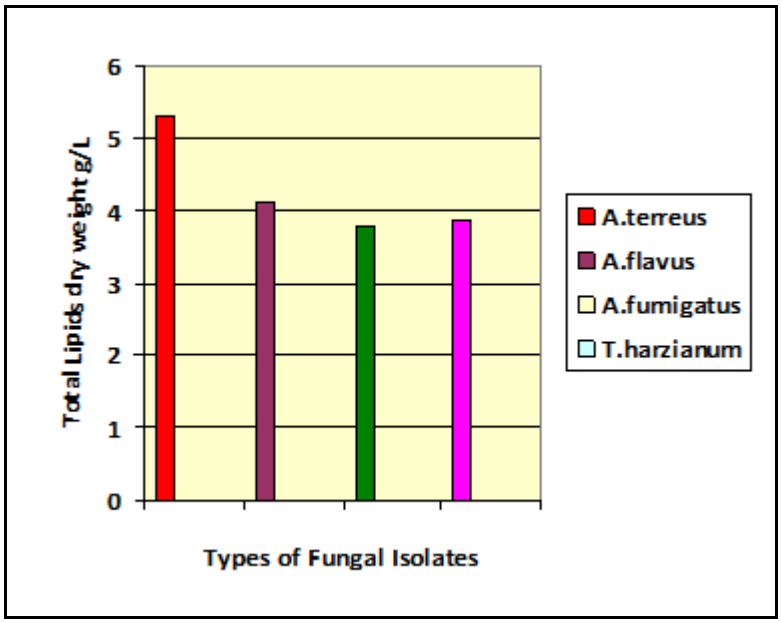

Figure 2. Total lipids dry weight in syrup date medium . pH 7and incubated for 7 days, 120 rpm at 30˚C.

The Lipids content of fungal isolates *A.flavus* , *A.fumigatus* , *A. terreus* , *T.harizanum* were found to be oleaginous fungi , as the total lipid contents were 20% or more of their dry weight. The development of techniques to produce single cell oil (SCO) by using oleaginous microorganisms such as fungi , had triggered significant attention [18.19] . These oleaginous fungi accumulate lipids, mostly in the form of Triaacylglycerols (TAG) which is consider as reserve compounds in all eukaryotic organisms like fungi, plants and animals,

several species of fungi are able to accumulate significant amounts of intracellular lipid [3].It is known that date of syrup is a mixture of monosaccharaides glucose about the ratio 35.5% and 27.9 % of fructose.

In addition to protein 1.2% and mineral salts 2.2% therefore this natural medium was considered a good medium for production of lipid because the best carbon source for lipid accumulation in fungi is glucose under limitation of nitrogen [20,21].

Biodiesel Production and Analysis by Gas Chromatography

Fatty acids composition FAMEs of the test oleaginous fungal isolates *A.terreus* in liquid medium syrup of date which were extracted by acid methanolysis during transesterfication process of fungal lipids extract to Fatty Acid Methyl Esters (FAMEs) and were determined by GC/ MS .As shown in figure-3, fatty acid profiles by GC/ MS showed the presence of hexadecanoic acid represented according to quantitative and qualitative analysis of FAMES was Palmitic acid 34 % which is the most common fatty acid (saturated)with an 16-carbon chain found in most fungi and it is a major component of the biodiesel thenOctadecenoic acid stearic acid is a saturated fatty acid with an 18-carbon chain, oleic acid C18 : 1n9c and linoleic acid C18 : 2n6c are unsaturated fatty acids ,mostly found in all oleaginous microorganisms which gave about 15 % the others types of fatty acids 11-Octadecenoic acid, methyl ester about 10% a few amounts of Tridecanoic acid , methyl ester C13-saturated fatty acid and Pythalic acid is adicarboxylic acid exhibiting percentage values of 3 % and 2 % respectively .the fatty acids profiles of *A.terreus* indicates the presence of both saturated and unsaturated forms of fatty acids in spite of the saturated forms tend to give more favorable properties of biodiesel ,but the optimized biodiesel should contain both saturated and unsaturated. Thus , the biodiesel quality is dependent on the fatty acid profile of the oil used as feedstock for its production Hexadecanoic acid and stearic acid was the most abundant fatty acid isolated from oleaginous fungi .This result was agreement with many studies [22,23,24 ,9] .

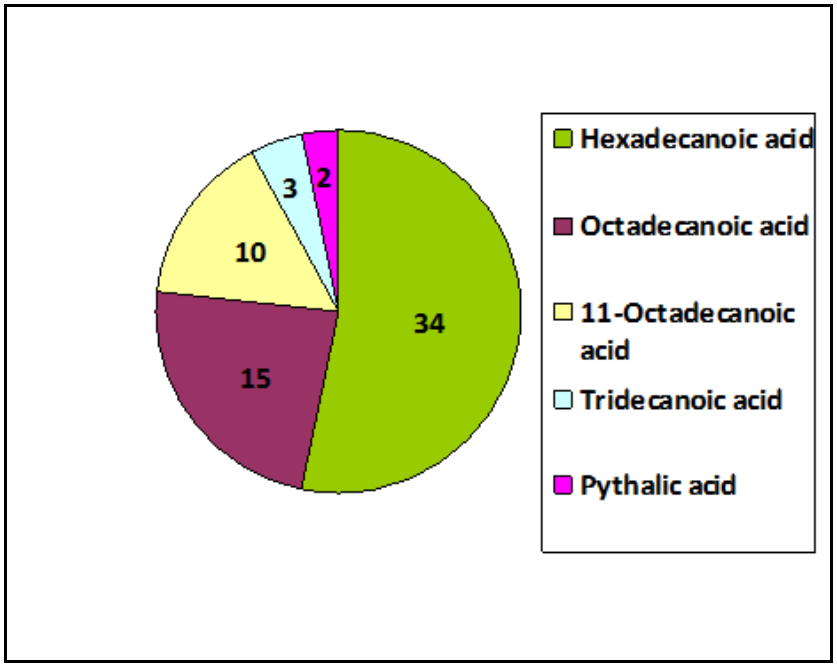

Figure 3: Fatty Acid Compositions and Percentage of the Extracted Total Lipids by GC/Mass of Fungal Test *Aspergillus terreus*

Lipids production on basal medium by fungal test *Aspergillus terreus* before and after mutation

In the present study it was found that the test fungal isolate *A.terreus* could produce high biomass of dry weight and total lipids production on the syrup date medium with increasing of exposing of UV light 5 min. and 10 min. of spore suspension of *A.terreus* as shown in Table 2,this may be due to increase lipolytic enzyme activity which mean increase accumulation of lipid intracellular cells of the test of fungal isolate *A. terreus*, which is a promising alternative oil for producing biodiesel. U.V irradiation was found to be for the improvement of stains of fungi *Rhizopus* sp.[25] and strain *A. niger* for maximum synthesis of lipase [26] . In recent years, attempts have been made for the overproduction of microbial enzymes by induced mutagenesis.

Prabakaran and others have reported that *Aspergillus fumigatus*, *Penicillium chrysogenum* and *Verticillium terrestre*, isolated from sugarcane field soil, Improvement in elucidation of amylase, cellulose and lipaseby developing mutants after exposure to UV light[27].

Table 2: Biomass dry weight (g/L) and Total Lipids dry weight (g/L) production on liquid medium by fungal test *Aspergillus terreus* before and after mutation when incubation at 30°C for 14 days.

Treatments	Biomass dry weight (g/L)	Total Lipids dry weight (g/L)	Total Lipids percentage to Biomass dry weight (%)
Before Mutation	10.22	5.35	52.34
After 5 min. of (U.V) light exposing	13.30	6.86	51.57
After 10 min. of (U.V)light exposing	17.53	9.75	55.61

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